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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS :	Gong, Kufe, Nicolette and Roberts		
SERIAL NUMBER :	09/782,492	EXAMINER :	Qian J. Li
FILING DATE :	February 12, 2001	ART UNIT :	1632
FOR :	CELL FUSIONS AND METHODS OF MAKING AND USING THE SAME		

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DECLARATION UNDER 37 C.F.R. § 1.132

I, DONALD KUFE, hereby declare and state as follows:

1. I received my Doctor of Medicine degree in 1970 at the University of Rochester School of Medicine. I am a named inventor on this application. I have been working in the fields of immunology, cell and molecular biology and with methods of treating cancer since 1977.

2. I understand that the pending claims are directed to substantially pure populations of educated, antigen-specific cytotoxic immune effector cells expanded in culture by contacting these effector cells with hybrid cells that are generated by fusion between at least one mammalian dendritic cell and at least one mammalian tumor or cancer cell.

3. I am aware of the Examiner's April 9, 2003 Office Action. In particular, I understand that the Examiner has rejected the pending claims under 35 U.S.C. § 102(e) contending that the pending claims are anticipated in view of United States Patent 6,306,388 to Nair *et al.* ("Nair"), and in view of United States Patent 6,156,307 to Granucci *et al* ("Granucci"). I understand the Examiner has also rejected the pending claims under 35 U.S.C. § 103(a) contending that the pending claims are obvious in view of Granucci in view of Moser *et al* (WO 96/30030) ("Moser"). According to the Examiner, the claims "are drawn to a substantially pure population of educated, antigen-specific immune effector cells, therefore, as long as Nair et al teach a population of such antigen specific immune effector cells, the claim limitations are met

regardless of how the cells are made, i.e., by culturing with hybrid cells or non-hybrid antigen-presenting cells." (Office Action at page 3). The Examiner also takes a similar position regarding Granucci, and the combination of Granucci and Moser.

4. I make this declaration to rebut the Examiner's rejection, with which I do not agree. I understand that the claims of the instant application have been amended to specify that, in addition to requiring substantially pure populations of educated, antigen-specific immune effector cells expanded in culture by contacting these effector cells with hybrid cells that are generated by fusion between at least one mammalian dendritic cell and at least one mammalian tumor or cancer cell, the pending claims also require that this population contains $CD4^+$ immune effector cells and $CD8^+$ cytotoxic immune effector cells. The hybrid cells of the instant application express shared and unique tumor-associated antigens, high levels of MHC class I and class II molecules, and adhesion and costimulatory molecules. (See, *e.g.*, page 2, lines 15-27; Figs. 1A, 4A-C, 8A-B, 9, 12A; and Parkhurst *et al.*, J. Immunol. 2003, 170:5317-25). Thus, the population of cytotoxic immune effector cells of the instant application, which are educated by these hybrid cells, contains both $CD4^+$ immune effector cells and $CD8^+$ cytotoxic immune effector cells, which, in my opinion, is a well-recognized advantage in a human cancer vaccine. It is well known in the art that $CD4^+$ immune effector cells and $CD8^+$ immune effector cells will interact with one another, thereby amplifying the resulting immune response. In fact, as shown in the instant application, T cells prepared according to the invention demonstrate "potent anti-tumor immunity." (See Specification, page 54, lines 9-10). Such populations of cytotoxic immune effector cells are neither taught nor suggested by Nair, Granucci, or by the combination of Granucci and Moser.

5. It is my opinion that the cytotoxic immune effector cells of the instant application are immunologically, biochemically, and patentably distinct from the immune effector cells that would result from using the methods of Nair. Nair teaches immune effector cells that have been contacted with antigen-presenting cells (APCs) that have been loaded with tumor cell-derived or pathogen-derived RNA. (See, *e.g.*, Nair col. 2, lines 55-60). Nair introduces RNA into the APCs, therefore endogenous proteins are presented on the cell surface. Thus, the populaton of immune effector cells educated by contact with the Nair APCs will be $CD8^+$, but

not $CD4^+$, because endogenous proteins are presented by Class I MHC molecules. For this reason, I believe that the population of cytotoxic immune effector cells of the instant application, which are generated by contact with hybrid cells formed by fusion of dendritic cells with cancer or tumor cells and contain both $CD4^+$ immune effector cells and $CD8^+$ cytotoxic immune effector cells, are distinct from the immune effector cells disclosed by Nair.

6. It is also my opinion that the population of cytotoxic immune effector cells of the instant application are immunologically, biochemically, and patentably distinct from the immune effector cells that would result from using the methods of Granucci. Granucci teaches immune effector cells that have been contacted with APCs that have been loaded with tumor antigens. (See, *e.g.*, Granucci col. 4, line 56 to col. 5, line 5). The immune effector cells disclosed by Granucci can be loaded with antigens that are associated with either Class I or Class II MHC molecules, but not both. (See, *e.g.*, Granucci col. 5, lines 19-34). Moreover, it is well known in the art that loaded polypeptide or protein antigens are presented by Class II MHC molecules only. Therefore, it is my belief that, because the population of immune effector cells generated by the method of Granucci will be limited to either $CD8^+$ or $CD4^+$, these immune effector cells are less useful as a cancer vaccine, as compared to the population of cytotoxic immune effector cells claimed here, which contains both $CD4^+$ immune effector cells and $CD8^+$ cytotoxic immune effector cells. It is well known that cytotoxic T cells require two signals to mature: the interaction with an MHC class I antigen, and IL-2, which is normally produced by nearby T helper cells. Granucci does not provide for either T helper cells or for another source of IL-2. Therefore, the effector cells produced by Granucci are not cytotoxic.

7. It is also my opinion that the population of immune effector cells of the instant application are immunologically, biochemically, and patentably distinct over the combination of Granucci and Moser. As stated above, the immune effector cells disclosed by Granucci can be loaded with antigens, which are associated with either Class I or Class II MHC molecules, but not with both. Moser discloses the fusion of immortal tumor cells from an autologous tumor with allogeneic dendritic-like cells. (See, *e.g.*, Moser Abstract). It is my belief that one of ordinary skill in the art seeking to overcome the limitations of Granucci discussed above would not be motivated to use the cell fusion technique of Moser-- in fact, Granucci teaches

that the use of fusions is not necessary in order to educate immune effector cells. However, even if, as asserted by the Examiner, one of ordinary skill in the art were motivated to use the fused cells of Moser in the process disclosed by Granucci, they would not obtain the population of cells containing both $CD4^+$ immune effector cells and $CD8^+$ cytotoxic immune effector cells of the instant application. Specifically, Moser was unable to obtain either murine (See, *e.g.*, Moser page 24, lines 1-2, and Table 1) or human (See, *e.g.*, Moser page 32, lines 14-15, and Table 2) fused cells that expressed B7 even though Moser used B7 expressing dendritic cells as a starting material (See, *e.g.*, Moser Tables 1 and 2). The B7 co-stimulatory polypeptide (also known as CD80) is critical to T cell activation. B7 expressed on APCs physically interacts with costimulatory polypeptide CD28 expressed on the surface of T cells. This interaction is crucial to T cell-APC communication that results in activation of T cells. The hybrid cells recited in the instant claims express B7. Therefore, these hybrid cells are more capable of educating T cells than cells that do not express this costimulatory polypeptide. Thus, for all the foregoing reasons, I believe that the pending claims are also not obvious over Granucci in view of Moser. Therefore, the Examiner should withdraw this rejection and allow the pending claims.

8. There are several advantages provided using the population of cytotoxic immune effector cells that have been contacted with hybrid cells generated by dendritic cell fusion to either tumor or cancer cells as claimed here, compared to the cited prior art. In my opinion, the immune effector cells disclosed in the instant application are superior to those prepared according to the methods of Granucci and Nair, and the combination of Granucci and Moser. The immune effector cells recited in the instant claims are cytotoxic, which is neither taught nor suggested by Granucci. Further, the claimed population of educated immune effector cells contains both $CD4^+$ immune effector cells and $CD8^+$ cytotoxic immune effector cells, while the population of educated immune effector cells of Nair can only contain $CD8^+$ immune effector cells, and the population of Granucci can only contain educated immune effector cells that are $CD4^+$ or $CD8^+$, but not both. Thus, for all the foregoing reasons, I believe that the pending claims are not anticipated by Nair or Granucci, or obvious in view of Granucci and Moser. Therefore, the Examiner should withdraw this rejection and allow the pending claims.

9. Moreover, it has become increasingly well-recognized that the educated immune effector cells created by fusion of dendritic cells fused to tumor or cancer cells is an important advance within the field of cancer biology. My 1997 paper, which is the scientific publication counterpart of the present application, discloses the induction of antitumor activity by immunization with fusions of dendritic cells and tumor cells and that blocking antibodies to CD4 or CD8 inhibit the immune response (See, *e.g.*, Figures 1, 2C and 2D, Gong *et al.*, Nat. Med. 1997, 3:558-561). This paper has been cited more than 220 times. Recently, other researchers have confirmed my results, showing that fusions of dendritic cells and tumor cells express both MHC class I- and class II-restricted tumor-associated epitopes, and, thus, are useful for the induction of tumor-reactive $CD4^+$ T cells and $CD8^+$ cytotoxic T cells. (See, *e.g.*, Parkhurst *et al.*, J. Immunol. 2003, 170:5317-25). Therefore, it has become clear that human dendritic cells fused to tumor cells have promise in human cancer vaccine trials.

10. I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001, Title 18, United States Code, and that willful false statements may jeopardize the validity of this application and any patent issuing therefrom.

Donald W. Kufe

Donald Kufe

Signed at Boston, MASSACHUSETTS
this 30th day of September 2003

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